

FORM PTO-1390 (Modified)
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

0273-0011

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

To Be Determined

10/089503

INTERNATIONAL APPLICATION NO.

PCT/EP00/09594

INTERNATIONAL FILING DATE

29-September-2000

PRIORITY DATE CLAIMED

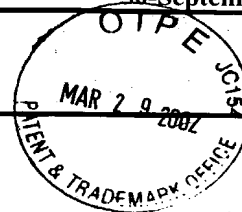
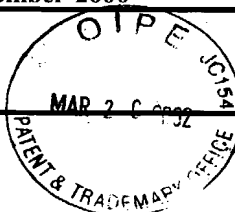
30-September-1999

TITLE OF INVENTION

THE PRV-1 GENE AND USE THEREOF

APPLICANT(S) FOR DO/EO/US

PAHL, Heike



Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☒ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

1. Statement to Support Filing and Submission of Sequence Listing in Accordance With 37 CFR 1.821-1.825; 2. English language translation of the Reply to the Written Notice containing Amended Claims, dated 15-October-2001; 3. Paper copy of the sequence listing (10 sequences); and 4. Postcard

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.101) To Be Determined		INTERNATIONAL APPLICATION NO. PCT/EP00/09594		ATTORNEY'S DOCKET NUMBER 0273-0011	
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24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :			CALCULATIONS PTO USE ONLY	
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	\$1040.00			
<input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	\$890.00			
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	\$740.00			
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	\$710.00			
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	\$100.00			
ENTER APPROPRIATE BASIC FEE AMOUNT =			\$890.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than _____ months from the earliest claimed priority date (37 CFR 1.492 (e)). <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30			\$130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	11 - 20 =	0	x \$18.00	\$0.00
Independent claims	8 - 3 =	5	x \$84.00	\$420.00
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>	\$0.00
TOTAL OF ABOVE CALCULATIONS =				\$1,440.00
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				\$0.00
SUBTOTAL =				\$1,440.00
Processing fee of \$130.00 for furnishing the English translation later than _____ months from the earliest claimed priority date (37 CFR 1.492 (f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30 +				\$0.00
TOTAL NATIONAL FEE =				\$1,440.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).			<input type="checkbox"/>	\$0.00
TOTAL FEES ENCLOSED =				\$1,440.00
			Amount to be refunded	\$
			charged	\$

a. ☒ A check in the amount of **\$1,440.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-0622**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

SHANKS & HERBERT
 TransPotomac Plaza
 1033 North Fairfax Street, Suite 306
 Alexandria, VA 22314
 (703) 683-3600
 (703) 683-9875 (facsimile)

SIGNATURE
Toni-Junell Herbert
 NAME
34,348
 REGISTRATION NUMBER
3/29/02
 DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: PAHL

Appl. No.: To Be Determined (U.S. National
Stage of PCT/EP00/09594)

Filed: Herewith

International Filing Date: September 29, 2000

For: The PRV-1 Gene and Use Thereof

Art Unit: To Be Determined

Examiner: To Be Determined

Atty. Docket: 0273-0011

**STATEMENT TO SUPPORT FILING AND SUBMISSION OF SEQUENCE
LISTING IN ACCORDANCE WITH 37 C.F.R. §§ 1.821-1.825**

Assistant Commissioner for Patents
Washington, D.C. 20231
Box SEQUENCE

Sir:

In connection with a Sequence Listing submitted concurrently herewith, the undersigned hereby states that:

1. the submission, filed herewith in accordance with 37 C.F.R. § 1.821(g), does not include new matter;
2. the content of the attached paper copy and the attached computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same as required by 37 C.F.R. § 1.821(f); and
3. all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these

Appl. No.: To Be Determined
(U.S. National Stage of PCT/EP00/09594
Atty. Docket No.: 0273-0011

10/089503
JC13 Rec'd PCT/PTC 29 MAR 2002

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Respectfully submitted,

SHANKS & HERBERT

By: Toni-Junell Herbert
Toni-Junell Herbert
Reg. No. 34,348

Date: 3/29/02

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03/06/2003 12:15 FAX 703 683 9875

SHANKS & HERBERT

10089503 042902
Rec'd PCT/PTO 06 MAR 2003

10/089503

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: PAHL, Heike

Art Unit: To Be Assigned

Appl. No.: 10/089,503 (U.S. National Stage of
PCT/EP00/09594)

Examiner: To Be Assigned

Filed: March 29, 2002 (International Filing Date:
Sept. 29, 2000)

Atty. Docket: 0273-0011

For: **The PRV-1 Gene and Use Thereof**

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants wish to thank Mr. Alverado of the PCT branch for his assistance during the teleconference today, March 5, 2003. During that conversation, Mr. Alverado requested that this preliminary amendment be filed to clarify the status of the claims. Specifically, because the amendment dated April 29, 2002 may have caused some confusion as to the status of the claims, applicants specifically request that the April 29, 2002 amendment not be entered. In its place, this preliminary amendment clarifies that 11 claims, now numbered 25-35, are pending in the application. Prior to examination, applicants respectfully request that the following amendment be entered:

IN THE CLAIMS:

Please cancel claims 1-24.

Please add the following new claims:

Received from < 703 683 9875 > at 3/6/03 11:17:09 AM [Eastern Standard Time]

U.S. Appl. No: 10/089,503
(U.S. National Stage of PCT/EP00/09594)
Atty. Dkt. No: 0273-0011

nucleotides 36-1238 of SEQ ID NO:1;
nucleotides 39-1346 of SEQ ID NO:1;
nucleotides 39-1262 of SEQ ID NO:1;
nucleotides 39-1238 of SEQ ID NO:1;
nucleotides 99-1346 of SEQ ID NO:1;
nucleotides 99-1262 of SEQ ID NO:1;
nucleotides 99-1238 of SEQ ID NO:1;
or a biologically active fragment thereof; or a biologically active variant thereof;
wherein the polynucleotide encodes a PRV-1 polypeptide or a functional fragment
or variant thereof and wherein the patient cell(s) express an effective amount of
the PRV-1 polypeptide or functional fragment or variant thereof.

28. A method for multiplying endogenous cells and/or established cell lines *ex vivo* or *in vitro*, comprising contacting the cells and/or cell lines with an effective amount of an N-glycosylated polypeptide comprising one of the following amino acid sequences:

amino acids 1-437 of SEQ ID NO:2;
amino acids 1-409 of SEQ ID NO:2;
amino acids 1-401 of SEQ ID NO:2;
amino acids 22-437 of SEQ ID NO:2;
amino acids 22-409 of SEQ ID NO:2;
amino acids 22-401 of SEQ ID NO:2;
or a biologically active fragment thereof comprising at least 50 amino acids; or a
biologically active variant thereof.

29. A method of inhibiting cell growth *in vivo* or *in vitro*, comprising contacting a cell(s) with a cell growth inhibiting amount of an N-glycosylated polypeptide comprising one of the following amino acid sequences:

amino acids 1-437 of SEQ ID NO:2;
amino acids 1-409 of SEQ ID NO:2;

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(U.S. National Stage of PCT/EP00/09594)
Atty. Dkt. No: 0273-0011

amino acids 1-401 of SEQ ID NO:2;
amino acids 22-437 of SEQ ID NO:2;
amino acids 22-409 of SEQ ID NO:2;
amino acids 22-401 of SEQ ID NO:2;
or a biologically active fragment thereof comprising at least 50 amino acids; or a
biologically active variant thereof.

30. The method of claim 29, wherein the polypeptide functions as a cytostatic agent.
31. A method for treating a proliferative disease in a patient, comprising administering to a patient in need thereof an N-glycosylated polypeptide comprising one of the following amino acid sequences:
amino acids 1-437 of SEQ ID NO:2;
amino acids 1-409 of SEQ ID NO:2;
amino acids 1-401 of SEQ ID NO:2;
amino acids 22-437 of SEQ ID NO:2;
amino acids 22-409 of SEQ ID NO:2;
amino acids 22-401 of SEQ ID NO:2;
or a biologically active fragment thereof comprising at least 50 amino acids; or a
biologically active variant thereof.
32. The method of claim 31, wherein the proliferative disease is selected from the group consisting of: a myeloproliferative disease, polycythemia rubra vera, essential thrombocythemia, myelofibrosis, CML, leukemia, a lymphoma and a solid tumor.
33. A method of inhibiting cell growth, comprising contacting a cell(s) with a polynucleotide comprising one of the following nucleotide sequences:
nucleotides 1-1600 of SEQ ID NO:1;
nucleotides 36-1346 of SEQ ID NO:1;

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(U.S. National Stage of PCT/EP00/09594)
Atty. Dkt. No: 0273-0011

nucleotides 36-1262 of SEQ ID NO:1;
nucleotides 36-1238 of SEQ ID NO:1;
nucleotides 39-1346 of SEQ ID NO:1;
nucleotides 39-1262 of SEQ ID NO:1;
nucleotides 39-1238 of SEQ ID NO:1;
nucleotides 99-1346 of SEQ ID NO:1;
nucleotides 99-1262 of SEQ ID NO:1;
nucleotides 99-1238 of SEQ ID NO:1;

or a biologically active fragment thereof; or a biologically active variant thereof;
wherein the polynucleotide encodes a PRV-1 polypeptide or a functional fragment or
variant thereof and wherein the cell(s) express a growth inhibiting amount of the PRV-1
polypeptide or functional fragment or variant thereof.

34. A method for treating a proliferative disease in a patient, comprising
administering to a patient in need thereof an N-glycosylated polynucleotide
comprising one of the following nucleotide sequences:

nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;

or a biologically active fragment thereof; or a biologically active variant thereof;
wherein the polynucleotide encodes a PRV-1 polypeptide or a functional fragment or
variant thereof and wherein the patient cell(s) express an effective amount of the PRV-1
polypeptide or a functional fragment or variant thereof.

U.S. Appl. No: 10/089,503
(U.S. National Stage of PCT/EP00/09594)
Atty. Dkt. No: 0273-0011

35. The method of claim 34, wherein the proliferative disease is selected from the group consisting of: a myeloproliferative disease, polycythemia rubra vera, essential thrombocythemia, myelofibrosis, CML, leukemia, a lymphoma and a solid tumor.


REMARKS

Claims 25-35 are pending in the above-identified application. Claims 1-24 have been canceled. Support for new claims 25-35 is found in canceled claims 1-24 and in the specification (English translation) at, for example, pages 12-14. No new matter has been added.

Applicant respectfully asserts that the application is now in condition for examination.

Respectfully submitted,

SHANKS & HERBERT

By: 
Shelly Guest Cermak
Reg. No. 39,571

Date: 3-5-03

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10/089503 10/089503
Rec'd PCT/PTO 29 APR 2002
10/089503

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Pahl, Heike

Serial No. 10/089,503 (U.S. National Stage of
PCT/EP00/09594

Filed: March 29, 2002 (International Filing Date:
Sept. 29, 2000

For: The PRV-1 Gene and Use Thereof

Art Unit: To Be Assigned

Examiner: To Be Assigned

Atty. Docket: 0273-0011

**AMENDMENT AND SUBMISSION OF SEQUENCE LISTING
UNDER 37 C.F.R. § 1.825(a)**

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. § 1.825(a), Applicants submit substitute sheets to
amend the paper copy of the Sequence Listing.

In the Specification:

Please cancel the existing Sequence Listing for the above-identified application,
and replace it with the substitute sheets appended hereto.

REMARKS

Applicants' Attorney hereby states that the submission, filed in accordance with
37 C.F.R. § 1.825(a), does not include new matter. Applicants' undersigned attorney has

amended the specification only to direct the entry of this corrected Sequence Listing between the specification and the claims of the above-identified application.

✓ In accordance with 37 C.F.R. 1.825(b), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith are the same.

Support for the new Sequence Listing is found in the canceled Sequence Listing found between the specification and claims of the above-identified application.

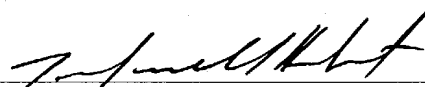
This amendment and submission is totally responsive to the Notification of Missing Requirements Under 35 U.S.C. 371 in the United States Designated/Elected Office (DO/EO/US). The Notice also requests an Oath or Declaration of the inventors. The Oath and Declaration were filed in the United States Patent and Trademark Office on April 20, 2002.

It is respectfully believed this application is now in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

SHANKS & HERBERT

By:



Toni-Junell Herbert

Reg. No. 34,348

Date:

9/20/02

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2/plyb

The gene PRV-1 and its use

Description

5 The invention relates to a nucleotide sequence
which encodes the PRV-1 gene, to recombinant DNA which
contains this nucleotide sequence, to vectors which
contain the recombinant DNA and to cells which are
transformed with these vectors, and also to a PRV-1
10 polypeptide, to antibodies against this polypeptide, to
a process for detecting the PRV-1 polypeptide and to
drugs which comprise the PRV-1 polypeptide or
antibodies which are directed against the PRV-1
polypeptide.

15 Polycythemia rubra vera (erythremia), also
termed polycythemia vera or p. vera, is a malignant
hematological disease in which there is an increased
formation of erythroid, granulocytic and megakaryocytic
cells. The disease is of clonal origin and arises as a
20 result of the mutation of a single hematopoietic
precursor cell. In Germany, the incidence of p. vera is
from 4 to 6 per million inhabitants. If left untreated,
the disease leads to death within 18 months. Treatment
by means of blood-letting or chemotherapy extends the
25 average survival time to more than 13 years.

P. vera is diagnosed by means of clinical
criteria. The clinical picture includes headaches,
pruritus, splenomegaly in two thirds of the patients,
bleeding or thromboses, hypertension in a third of the
30 patients, gout, which is brought about by an increase
in the production of uric acid, and, in some cases,
septic ulcers. The most important laboratory finding is
an increase in the values for hemoglobin, hematocrit,
erythrocyte count and total erythrocyte volume, and
35 also a neutrophilic granulocytosis or thrombocytosis in
many cases. Since, on the one hand, most of the
criteria are rather diffuse and, on the other hand, not
all the patients fulfill these criteria, it is

frequently difficult to distinguish p. vera from other myeloproliferative diseases, such as chronic granulocytic leukemia or essential thrombocytosis, and thereby confirm the diagnosis. To date, the molecular
5 cause of p. vera is completely unknown. Since, however, p. vera takes a severe course if it is not treated, accurate diagnosis is important.

An object of the invention was therefore to find the molecular cause of polycythemia rubra vera and
10 to create the possibility of diagnosing it.

This object was achieved by isolating a gene which is expressed specifically in association with p. vera and not in healthy control individuals. This gene is designated the PRV-1 gene (polycythemia rubra vera).

15 A similar nucleotide sequence is disclosed in International application WO 98/50552.

One part of the subject matter of the invention therefore relates to a polynucleotide which encodes the PRV-1 gene and essentially comprises the sequence ID
20 No. 1. The polynucleotides of the present invention can be single-stranded or double-stranded DNA or RNA. If they are RNA, it is then clear to the skilled person that "U" nucleotides are present in place of "T" nucleotides. "Polynucleotide" is understood as meaning
25 nucleic acids which contain 15 or more nucleotides.

The nucleotide sequence according to the invention is depicted in figure 1. The invention therefore relates to a polynucleotide which corresponds to the sequence shown in figure 1 and also to a
30 polynucleotide whose nucleotide sequence exhibits minor differences. Within the meaning of the present application, minor differences are understood as meaning those sequences in which a few, preferably not more than 50 and particularly preferably not more than
35 25, nucleotides can be exchanged, with, however, the function of the gene encoded by the nucleotide sequence being unaffected. The skilled person is familiar with the fact that a base triplet encoding an amino acid can be replaced with another triplet which encodes the same

amino acid. In addition to this, regions which are of less importance can be deleted and/or mutated to a minor extent. In a particular embodiment, the polynucleotide comprises nucleotides 36 to 1346 of sequence No. 1, that is the coding region of the PRV-1 gene. Other embodiments comprise nucleotides 36 to 1262 or 36 to 1238 of sequence No. 1. This region presumably encodes the active region of the PRV-1 polypeptide. Finally, the polynucleotide of the invention can also comprise nucleotides 39 to 1346, 39 to 1262 or 39 to 1238 of sequence No. 1, such that the codon which encodes the starting methionine is not present. A preferred embodiment is a polynucleotide which comprises nucleotides 99 to 1346, 99 to 1262 or 99 to 1238 of sequence No. 1. This results in the codons at the 5' end which encode the signal peptide of the PRV-1 polypeptide not being present.

The polynucleotide according to the invention can also be a fragment of the PRV-1 gene. As a rule, the fragment possesses more than 100 nucleotides, preferably, however, more than 300 nucleotides. The fragments can also be used as primers or as probes, in particular for PCR; in this case, the fragments can be truncated to fit the purpose. Usually, primers have a length of between 10 and 30 nucleotides and probes have a length of between 15 and 50 nucleotides.

The PRV-1 gene is an endogenous gene whose expression in healthy individuals is, however, restricted to only a few organs. Normally, it is expressed in the main in the hematopoietic organs, i.e. in bone marrow and fetal liver, and weakly expressed in the spleen, but not expressed in heart, muscle, pancreas or kidney. In patients who are suffering from p. vera, this gene is very strongly overexpressed in the hematopoietic cells, in particular.

The PRV-1 gene encodes a protein which exhibits the protein sequence shown in Figure 2. The signal peptide, which is present in the protein sequence of all surface molecules and normally removed when the

protein is processed, is divided off by a hyphen. The protein has the sequence ID No. 2. Another aspect of the invention is consequently an essentially pure polypeptide having the sequence No. 2 or a polypeptide having the sequence No. 2 but lacking the signal peptide (i.e. amino acids 22 to 437 of sequence No. 2). Other embodiments encompass amino acids 1 to 409, 22 to 409, 1 to 401 or 22 to 401 of sequence No. 2 (what is probably the active region of the protein).

With regard to biological activity, the polypeptide according to the invention is preferably glycosylated; it is most preferably N-glycosylated. It can then be glycosylated at at least one of the amino acids Asn-46, Asn-189 and Asn-382 of the PRV-1 polypeptide (the amino acid numbers refer to the sequence No. 2). The invention also encompasses fragments of the polypeptides according to the invention which are N-glycosylated. The fragments are at least 50 amino acids in length, preferably at least 100 amino acids and most preferably at least 150 amino acids. In another embodiment, a polypeptide can be O-glycosylated.

It is clear to the skilled person that particular amino acids can be replaced with other amino acids without impairing the biological activity of the protein. Such modified forms of the polypeptides according to the invention are also part of the subject matter of the invention (variants). The amino acid replacements are those which do not have a negative effect on the biological activity of the protein. The skilled person can make use of well known rules for selecting the replacements.

Depending on the method of preparation, the PRV-1 polypeptide can, for example, possess a glycosyl phosphatidylinositol anchor. This is then bonded to the amino acids which correspond to amino acids 407 to 409 in sequence ID No. 2. A GPI anchor is used to anchor a protein by means of a lipid on the outside of the cell membrane. However, for reasons which have not so far

been conclusively elucidated, it is frequently observed that GPI-linked proteins are also released into the medium. This is referred to as "shedding". To date, it has not been clarified whether this is a specific process, i.e. such proteins are cleaved from the membrane by enzymes in a controlled manner, or whether it represents a non-specific loss of the anchor. It is consequently very probable that PRV-1 is to be found both on the cell membrane and extracellularly. The secreted form, which is not membrane-bound, is probably more important for the effect of the polypeptide as a growth factor and growth inhibitor since, as a growth factor, this form is able to diffuse and reach other cells.

It is clear to the skilled person that he can influence the attachment of the protein to the cell membrane by manipulating these C-terminal amino acids. This particularly concerns the preparation of defined DNA constructs which are intended for expressing the PRV-1 polypeptide or fragments of this polypeptide. The codons which encode these amino acids can be mutated or deleted.

The gene encodes a surface receptor of the uPAR/Ly6 family. This receptor family can transduce mitogenic signals, i.e. signals which stimulate cell division. It is therefore assumed that overexpression of the PRV-1 gene, inter alia on the bone marrow cells of p. vera patients, contributes to hyperproliferation of these cells.

It has been found that PRV-1 is not expressed on granulocytes in healthy individuals or in patients suffering from other myeloproliferative diseases, e.g. suffering from chronic granulocytic leukemia, acute granulocytic leukemia, essential thrombocytosis or secondary erythrocytosis.

In order to be able to use the polypeptide encoded by the PRV-1 gene for analyses and detection methods, it is expediently generated from recombinant DNA, with the recombinant DNA preferably comprising the

nucleotide sequence ID No. 1 or at least the coding region of the PRV-1 gene, that is nucleotides 36 to 1346 of sequence ID No. 1, or else at least nucleotides 39 to 1262 or 39 to 1238, functionally linked to a promoter. However, the recombinant DNA can also
5 comprise only a fragment of sequence No. 1.

The invention furthermore relates to a vector which contains the recombinant DNA for the PRV-1 polypeptide, or a fragment thereof, and to a host cell
10 which is transfected or transformed with this vector. The host cells may be prokaryotic, for example bacteria such as E. coli. However, the polypeptides which are expressed are then not glycosylated. Preference is therefore given to eukaryotic host cells, which are
15 able to glycosylate the expressed protein post-translationally and modify it in other ways. Examples of eukaryotic host cells are insect cells, such as Sf9 cells, for expression following infection with recombinant baculoviruses, and mammalian cells, such as
20 293 cells, COS cells, CHO cells and HeLa cells. These examples are not exhaustive. It is also possible to use yeast cells as host cells. It is clear to the skilled person that the glycosylation pattern can differ depending on the host cell. The biological activity of
25 the expression product can therefore also vary. Particular preference is given to host cells which glycosylate the expression product in such a way that the biological activity of the protein is retained.

Another aspect of the invention is a process
30 for preparing a polypeptide according to the invention. In this process, a DNA encoding the polypeptide according to the invention is caused to be expressed in a host cell. The culture medium or the cells is/are employed for the subsequent isolation of the
35 polypeptide depending on whether the expressed polypeptide is secreted by the host cell into the culture medium or remains in the cell. After that, the polypeptide according to the invention is concentrated and/or purified using methods which are known in the

which can be used as probes in the Northern blot, can be prepared by digesting the gene with restriction endonucleases. If the fragments are derived from the cDNA, they are then present as double strands which have to be separated into the single strands for the hybridization. Suitable examples are the Bam HI-PstI fragment from base pair 420 to base pair 831, or the PstI-PstI fragment from base pair 831 to base pair 1900.

PRV-1 mRNA, and consequently the expression of PRV-1, can also be detected by first of all reverse-transcribing the mRNA in an RT-PCR reaction and then amplifying the cDNA; the amplified DNA is then detected with a probe in a hybridization method.

In the case of a positive diagnosis, the disease has to be treated since it otherwise leads to death within a relatively short period of time. For this treatment, it is possible to use specific antibodies which are directed against PRV-1 and to which cytotoxic components can be bonded, where appropriate.

The invention therefore furthermore relates to a drug which, in addition to the customary excipients, comprises antibodies which are directed against the PRV-1 receptor.

Since the PRV-1 receptor is overexpressed in p. vera, many antibodies are bound on the surface of the affected granulocytes when they come into contact with the anti-PRV-1 antibody. The binding of many antibodies to these cells stimulates the immunological cells to destroy these granulocytes. In this way, it is possible to eliminate the p. vera cells specifically.

Surprisingly, it has also been found that the PRV-1 polypeptide exhibits haematopoietic activity. The PRV-1 polypeptide is able to stimulate certain hematopoietic precursor cells to form erythroid colonies. It is particularly the N-glycosylated PRV-1 polypeptides which display this function. The polypeptides according to the invention which are

preferred are therefore the N-glycosylated PRV-1 polypeptides, and fragments thereof, which display the growth factor activity.

Another aspect of the invention is therefore a
5 drug which, in addition to a pharmaceutically tolerated excipient, comprises the PRV-1 polypeptide or a biologically active fragment thereof. The PRV-1 polypeptide is preferably glycosylated PRV-1 polypeptide and, even more preferably, N-glycosylated
10 PRV-1 polypeptide or a biologically active fragment thereof. The invention also relates to drugs which comprise at least one polynucleotide according to the invention.

The present invention furthermore relates to
15 the use of PRV-1 polypeptide, or a biologically active fragment thereof or a biologically active variant thereof, as a growth factor in vivo and ex vivo. The PRV-1 polypeptide, or a biologically active fragment thereof or a biologically active variant thereof, can
20 be used for treating all pancytopenias and pancytopathies in the bone marrow and in the circulation (change in the cellular constituents of the peripheral blood and bone marrow). The polypeptides of the present invention can, for example, be used for
25 treating anemias in the case of kidney failure, chemotherapy or whole body radiation, for treating neutropenias and thrombocytopenias during chemotherapy or whole body radiation, for the ex-vivo treatment of peripheral or bone marrow stem cells for expansion
30 (multiplication) and retransfusion into the patients, and for treating sepsis, systemic inflammatory response syndrome (SIRS) or regional inflammatory reactions. The polypeptides of the present invention, or drugs which comprise them, can be administered in a wide variety of
35 ways. The forms of administration comprise intravenous, intramuscular, subcutaneous, intraperitoneal, oral, transdermal and transmucosal administration.

The polynucleotides according to the invention can also be used for treating pancytopenias and

pancytopathies. In this case, the aim is to express a PRV-1 polypeptide, or a functional fragment thereof, in cells of the affected patient. Gene therapy methods are first and foremost used in this connection. Cells can
5 be isolated from the patient and transfected with a polynucleotide according to the invention (ex-vivo manipulation), after which they are then returned to the patient. It is also possible to conceive of methods in which the polynucleotides according to the invention
10 gain access into the target cells by means of viral transfer. Expression of the inserted nucleic acids then leads to haematopoietic activity.

Surprisingly, it was also found that, at higher concentration, the PRV-1 polypeptide has an inhibitory
15 effect on the growth of cells. Thus, it was observed, for example, that adding an increased quantity of PRV-1 protein virtually completely stops the formation of erythroid and granulocytic/monocytic colonies. This effect resembles the action of Interferon- α , which is
20 used, inter alia, therapeutically in chronic myeloid leukemia (CML) and in p. vera. An endogenous inhibitory substance possesses great advantages as compared with a chemical cytostatic agent, such as hydroxyurea, which was used when Interferon- α was not yet available and is
25 to some extent still used. A disadvantage of Interferon- α is that this active compound has very severe side effects. The patients feel as if they were suffering from a serious influenza. The present invention makes available a hematopoiesis-inhibiting
30 substance, with the inhibitory activity being concentration-dependent.

Another aspect of the invention is therefore the use of a PRV-1 polypeptide, as described in this application, for inhibiting the growth of cells, in
35 particular its use as a cytostatic agent. Preference is given to the polypeptide being used for inhibiting the growth of hematopoietic cells. The invention also relates to the use of a polypeptide according to the invention for producing a drug for treating

proliferative diseases. These diseases are, in particular, the myeloproliferative diseases, p. vera, essential thrombocythemia, myelofibrosis, CML and also all leukemias and lymphomas and also solid tumors.

5 Another aspect of the invention is the use of a polynucleotide, as described in this application, of a biologically active fragment or of a biologically active variant thereof, for inhibiting the growth of cells. The polynucleotide can be incorporated into a
10 suitable vector and transfected into suitable target cells. After the PRV-1 polypeptide, or a biologically active fragment thereof, or a biologically active variant thereof, has been expressed in an appropriate concentration, the growth-inhibiting effect comes into
15 operation. In the same way, the polynucleotide can be incorporated into a viral vector, after which appropriate target cells are infected virally, leading to PRV-1 being expressed. The invention also relates to the use of a polynucleotide of this application for
20 producing a drug for treating proliferative diseases, such as the myeloproliferative diseases, p. vera, essential thrombocythemia, myelofibrosis, CML and also all leukemias and lymphomas and also solid tumors.

The invention also relates to kits for
25 detecting either polycythemia vera or disturbances of the hematopoietic system. These kits comprise a polynucleotide according to the invention and/or a polypeptide according to the invention and/or one or more antibodies according to the invention. In addition
30 to this, the kit can also comprise a container or compositions which are suitable for implementing detection reactions. Examples of such compositions are buffer solutions, reagents for blocking membranes, hybridization solutions, secondary antibodies,
35 substrate solutions for detection reactions, etc. The kit is preferably used for implementing PCR reactions, RT-PCR, Northern blots, Southern blots, Western blots and ELISA, RIA or similar reactions.

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The following examples are given in explanation.

Example 1

5

Characterizing the PRV gene

The following experiments were carried out in order to characterize the gene:

- 10 - the following protocol was used to isolate granulocytes from stored blood or from blood obtained by bleeding p. vera patients:
- an equal volume of 3% dextran solution in 0.9% NaCl was added to the blood and the mixture was left to
- 15 stand at room temperature (RT) for 20 minutes.
- The mixture separated into two phases. The upper, light-colored phase was removed and centrifuged for 10 minutes at 1800 g and at RT.
- The supernatant was discarded and the cell
- 20 pellet was resuspended in the same volume of 0.9% NaCl.
- In each case 35 ml of the cells in NaCl were layered on 15 ml of Ficoll-Hypaque.
- The cells on the Ficoll-Hypaque were then centrifuged for 60 minutes at 1800 g and at RT without
- 25 using the brake.
- A cell pellet and two layers with an interphase were formed.
- The layers and interphase were aspirated off and the cell pellet was resuspended for 30 seconds in
- 30 10 ml of ice-cold 0.2% NaCl, and 10 ml of ice-cold 1.6% NaCl were added immediately after 30 seconds.
- The cells were centrifuged down for 10 minutes at 1800 g and at RT.
- They were then washed once in 10 ml of PBS and
- 35 centrifuged down.
- The cell pellet contained 95-99%-pure granulocytes.
- RNA was isolated from these cells using standard methods.

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- 10 mg of this RNA were examined for the expression of PRV-1 in a Northern blot. The entire cDNA sequence shown in SEQ ID No. 1 was used as a probe.

5 This experiment was performed on 39 p. vera patients and 29 control samples of stored blood. The PRV-1 probe was found to hybridize strongly in the case of the p. vera patients. No hybridization was observed in healthy control samples.

10 Example 2

PRV-1 possesses growth factor activity

15 Embryos were removed from a pregnant mouse 13.5 days after fertilization. The fetal livers were removed. The cells contained in them were stained using antibodies and enriched for particular cells, and depleted for other cell types, by means of column chromatography. This results in a cell mixture which is
20 enriched for certain hematopoietic precursor cells (colony forming units-erythroid, CFU-E). Thus, while in all approximately 2% of the fetal liver consists of CFU-E, 30-40% of the enriched cells consist of CFU-E.

25 These CFU-Es were transfected using a retrovirus. To do this, a packaging cell line, designated 293-T, was itself transfected 48 hours previously. 293-T cells are an established human embryonic kidney cell line. 293-T cells are stably transfected with several genes from a retrovirus. If
30 these 293-T cells are now transfected with two plasmids, termed pOS and pKAT, the 293-T cells then produce a retrovirus which is able to infect murine fetal liver cells. If the 293-T cells are transfected with an empty pOS vector and pKAT, a wild-type
35 retrovirus, which only expresses retroviral proteins, is then produced. On the other hand, cloning a human gene, e.g. PRV-1, into the pOS vector results in the production of a retrovirus which expresses this protein

vector, without pKAT, no retroviruses are then formed. The cell culture medium then only contains the soluble protein produced by the cells. Medium which is derived from pOS-PRV-1-transfected cells, and which does not
5 contain any retrovirus, is mixed with CFU-Es and the whole is plated out in the methyl cellulose medium; the resulting colonies are then counted.

The following results were obtained:

10 Table 2: Solubility of PRV-1. The figures in each case indicate the number of colonies.

	Mixture 1	Mixture 2	Mixture 3	Mixture 4
	un- transfected	empty vector (pOS)	GFP (pOS-GFP)	PRV-1 (pOS-PRV-1)
Experiment 4		137	187	557

In this experiment, too, CFU-Es which were
15 treated with PRV-1-containing medium formed very many more hematopoietic colonies than did control cells. It can be concluded from this result that PRV-1 is a soluble growth factor.

20 Example 4

PRV-1 also has an inhibitory, cytostatic effect.

The experiments were carried out on peripheral
25 blood cells. Since a small number of precursor cells are also circulating in the peripheral blood in healthy individuals, it is possible to culture hematopoietic colonies from peripheral blood cells in a suitable medium (methyl cellulose). 40 ml of peripheral venous
30 blood were withdrawn from a healthy donor (while initially introducing heparin or EDTA as an anticoagulant). 15 ml of Ficoll/Hypaque were added to the blood and the mixture was centrifuged at 1 600 rpm for 40 minutes without braking. This results in the
35 production of a density gradient which fractionates the

IMDM medium, which had been incubated for 48 hours with untransfected cells (293), or in medium which had been incubated for 48 hours with cells which were expressing the altered PRV-1 (293-GPI-less-PRV-1). The ability of these cells to form hematopoietic colonies was then investigated. The number of erythroid (red) and myeloid (white) blood cell colonies was determined after 14 days. The experiment was repeated three times and also carried out on different days and using different blood donors. Duplicates were also evaluated within the experiment. The following results were obtained:

Experiment 1

Cell supernatant	Donor 1		Donor 2	
	Red colonies	White colonies	Red colonies	White colonies
293	248/221	70/114	127/161	25/66
293-GPI-less-PRV-1	7/3	0/0	31/19	0/0

Experiment 2

Cell supernatant	Donor 1		Donor 2	
	Red colonies	White colonies	Red colonies	White colonies
293	99/91	20/19	49/33	8/1
293-GPI-less-PRV-1	0/0	0/0	0/0	0/0

Experiment 3

Cell supernatant	Donor 1		Donor 2	
	Red colonies	White colonies	Red colonies	White colonies

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293	107/207	22/30	24/32	5/8
293-GPI-less- PRV-1	4/3	0/6	0/1	3/0

It can be concluded from this data that a higher dose of PRV-1 than that used in example 3 possesses a cytostatic effect.

5

Example 5

The growth factor PRV-1 is N-glycosylated

10 Granulocytes were isolated from a patient suffering from p. vera, and protein extracts were prepared from these cells using a standard protocol. These protein extracts were treated in accordance with the protocol for the "N-Glycosidase F Deglycosylation
15 Kit" supplied by Boehringer Mannheim. In detail, this means that a "denaturation buffer" was added to the protein extracts and the mixtures were heated at 95°C for 3 minutes, after which they were treated either with "reaction buffer" or with "reaction buffer" plus
20 N-glycosidase. Each mixture was incubated overnight at 37°C and the proteins were analyzed on a PAGE gel electrophoresis followed by a Western blot. The PRV-1 protein was detected with an antibody directed against a protein having the amino acid sequence ID No. 5. The
25 results show that while PRV-1 protein purified from granulocytes is 60-65 kDa in size, it is only 40 kDa in size after having been digested with N-glycosidase. This clearly proves that PRV-1 is glycosylated on asparagine residues (asparagine = N).

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Patent claims

- 5 1. An N-glycosylated polypeptide, essentially
comprising one of the following amino acid
sequences:
- 10 amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;
amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2;
or a fragment thereof containing at least 50 amino
15 acids.
2. A polypeptide, essentially consisting of one of
the following amino acid sequences:
- 20 amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2.
3. An antibody against a polypeptide of claim 1.
- 25 4. An antibody as claimed in claim 3, characterized
in that it is a monoclonal antibody.
5. A process for detecting polycythemia vera,
30 characterized in that the PRV-1 polypeptide is
reacted, in an immunoassay, with one or more
antibodies as claimed in claim 3 or 4.
6. A process as claimed in claim 5, characterized in
35 that the antibody employed is a polyclonal or
monoclonal antibody as claimed in claim 3 or 4.
7. A drug for treating polycythemia vera,
characterized in that, in addition to customary

excipients, it comprises antibodies as claimed in claim 3 or 4.

8. A drug which comprises a polypeptide as claimed in
claim 1 or a polypeptide which essentially
comprises one of the following amino acid
sequences:
- amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;
amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2;
and at least one pharmaceutically tolerated
excipient.
9. A drug which comprises a polynucleotide which
essentially comprises one of the following
nucleotide sequences:
- nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;
and at least one pharmaceutically tolerated
excipient.
10. The use of a polypeptide as claimed in claim 1 or
of a polypeptide which essentially comprises one
of the following amino acid sequences:
- amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;

9. A drug which comprises a polynucleotide which essentially comprises one of the following nucleotide sequences:
- 20 nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
- 25 nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;
- 30 and at least one pharmaceutically tolerated excipient.

10. The use of a polypeptide as claimed in claim 1 or
of a polypeptide which essentially comprises one
35 of the following amino acid sequences:
- amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;

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amino acids 22-409 of sequence No. 2;
amino acids 22-401 of sequence No. 2;
or of a biologically active fragment thereof or a
biologically active variant thereof, as a growth
factor.

11. The use of a polypeptide as claimed in claim 1 or
of a polypeptide which essentially comprises one
of the following amino acid sequences:
- 10 amino acids 1-437 of sequence No. 2;
amino acids 1-409 of sequence No. 2;
amino acids 1-401 of sequence No. 2;
amino acids 22-437 of sequence No. 2;
amino acids 22-409 of sequence No. 2;
15 amino acids 22-401 of sequence No. 2;
or of a biologically active fragment thereof or a
biologically active variant thereof, for producing
a drug for treating pancytopenias and
pancytopathies in the bone marrow and in the
20 circulation.

12. The use of a polynucleotide which essentially
comprises one of the following nucleotide
sequences:
- 25 nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
30 nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
nucleotides 99-1238 of sequence No. 1;
35 or of a fragment thereof or a variant thereof, for
producing a drug for treating pancytopenias and
pancytopathies in the bone marrow and in the
circulation.

amino acids 22-401 of sequence No. 2;
or of a biologically active fragment thereof or a
biologically active variant thereof, for producing
a drug for treating proliferative diseases.

5

17. The use as claimed in claim 16, characterized in
that the proliferative disease is selected from
the group comprising myeloproliferative diseases,
p. vera, essential thrombocythemia, myelofibrosis,
10 CML, all leukemias and lymphomas and also solid
tumors.

18. The use of a polynucleotide which essentially
comprises one of the following nucleotide
15 sequences:

nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
nucleotides 36-1238 of sequence No. 1;
20 nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;
nucleotides 99-1262 of sequence No. 1;
25 nucleotides 99-1238 of sequence No. 1;

or of a fragment thereof or a variant thereof, for
inhibiting the growth of cells.

19. The use of a polynucleotide which essentially
comprises one of the following nucleotide
30 sequences:

nucleotides 1-1600 of sequence No. 1;
nucleotides 36-1346 of sequence No. 1;
nucleotides 36-1262 of sequence No. 1;
35 nucleotides 36-1238 of sequence No. 1;
nucleotides 39-1346 of sequence No. 1;
nucleotides 39-1262 of sequence No. 1;
nucleotides 39-1238 of sequence No. 1;
nucleotides 99-1346 of sequence No. 1;

or a biologically active fragment thereof or a
biologically active variant thereof
and/or
at least one antibody as claimed in claim 3 or 4.

5

22. A kit for detecting the PRV-1 protein as claimed in
claim 21, characterized in that it is an ELISA test
kit.

10

23. A kit as claimed in claim 21 for detecting PRV-1
mRNA, characterized in that it is a
semiquantitative or quantitative RT-PCR analysis
kit.

15

24. A kit as claimed in claim 21 for detecting
PRV-1 mRNA, characterized in that it is a Northern
blot kit.

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Abstract

This document describes a nucleotide sequence which encodes the PRV-1 protein, and essentially comprises the sequence ID No. 1, and also a process for detecting this gene [lacuna] the mRNA coded by this gene and the polypeptide encoded by this gene.

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AAAAGCAGAAAGAGATTACCAGCCACAGACGGGTCAAGAGCGGGTATTACTGCTGGCCCTCC
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GGGGAGAGCCTGGAGCATCCGGAATTGCCCTATGGGAGAGGGGACGCTGGAGGAGTGGCTGCA
TGTATCTGATAATACAGACCCTGTC

Fig. 1

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MSAVLLLALLGFILPLPGVQA---LLCQFGTVQHVKVSDLPRQWTPKNTSCD
SGLGCQDTLMLIESGPQVSLVLSKGCTEAKDQEPRVTEHRMGPGLSLISY
TFVCRQEDFCNNLVNSLPLWAPQPPADPGSLRCPVCLSMEGCLEGTTEEI
CPKGTTHCYDGLLRRLRGGGIFS NLRVQGCMPPGCGNLLNGTQEIGPVGMT
ENCNRKDFLTCHRGTTIMTHGNLAQEPTDWTTSENTEMCEVGQVCQETLLL
IDVGLTSTLVGTKGCSTVGAQNSQKTTIHSAPPGVLVASYTHFCSSDLN
SASSSSVLLNSLPPQAAPVPGDRQCPTCVQPLGTCSSGSPRMTCPRGATH
CYDGYIHLSGGGLSTKMSIQGCVAQPSSFLLNHTRQIGIFSAREKRDVQP
PASQHEGGGAEGLES LTWGVGLALAPALWVGVCPCSC

Fig. 2

As a below named inventor, I hereby declare that:

_____ is attached hereto.
 X was filed on September 29, 2000 ✓
as United States Application Number _____
or PCT International Application Number PCT/EP00/09594 ✓

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above. I do not know and do not believe that the claimed invention was ever known or used in the United States of America before my invention thereof, or patented or described in any printed publication in any country before my invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, and that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months (for a utility patent application) or six months (for a design patent application) prior to this application.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d), of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Priority
Claimed

<u>19947010.3</u> ✓ (Number)	<u>DE - Germany</u> ✓ (Country)	<u>30-September-1999</u> ✓ (Day/Month/Year Filed)	<u>X</u> Yes	<u> </u> No
<u>PCT/EP00/09594</u> ✓ (Number)	<u>WO - PCT</u> ✓ (Country)	<u>29-September-2000</u> ✓ (Day/Month/Year Filed)	<u>X</u> Yes	<u> </u> No
<u> </u> (Number)	<u> </u> (Country)	<u> </u> (Day/Month/Year Filed)	<u> </u> Yes	<u> </u> No

(Application Number)

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SEQUENCE LISTING

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 Trp Lys Val Ser Asp Leu Pro Arg Gln Trp Thr Pro Lys Asn Thr Ser
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 Cys Asp Ser Gly Leu Gly Cys Gln Asp Thr Leu Met Leu Ile Glu Ser
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 Gly Pro Gln Val Ser Leu Val Leu Ser Lys Gly Cys Thr Glu Ala Lys
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 Leu Ile Ser Tyr Thr Phe Val Cys Arg Gln Glu Asp Phe Cys Asn Asn
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 Asp Gly Tyr Ile His Leu Ser Gly Gly Gly Leu Ser Thr Lys Met Ser
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